Int. J. Indust. Entomol. Vol. 24, No. 1, 2012, pp. 41~47

[Note]

International Journal of *Industrial Entomology*

COII Sequence-based Study for Population Genetic Variation of a Ground Beetle, *Scarites aterrimus* (Coleoptera: Carabidae)

Ah Rha Wang and Iksoo Kim*

College of Agriculture & Life Sciences, Chonnam National University, Gwangju 500-757, Korea

(Received 21 February 2012; Accepted 11 March 2012)

The *Scarites aterrimus* (Coleoptera: Carabidae) dwells exclusively on coastal sandy dunes. Previously, we investigated the nation-wide magnitude and nature of genetic diversity of the species using mitochondrial COI gene and found moderate to low magnitude of sequence diversity, the presence of closely related haplotypes, and relatively high gene flow estimate. Based on these observations we concluded that the species had no historical barriers that bolster genetic subdivision and possible population decline. In this study, we additionally sequenced mitochondrial COII gene from 23 individuals collected from 9 Korean localities to confirm previous findings. Sequencing of 688 bp COII gene provided 5 haplotypes ranging in sequence divergence from 0.145% to 0.291% (1 ~ 2 bp), further confirming low sequence divergence of the species. Gene flow estimates and genetic diversity estimates also support the previous findings that there had been no historical barriers that bolster genetic subdivision.

Key words: Mitochondrial DNA, COII gene, *Scarites aterrimus*, Ground beetle, Carabid Sequence divergence, Population genetic structure

Introduction

The ground beetle, *Scarites aterrimus* (Carabidae: Coleoptera), is one of the carabids well adapted to coastal duns (Kawakami *et al.*, 2006). Due to the habitat diminishment population decline was concerned for the carabid beetles including *S. aterrimus* (Kim, 1980, 2003). In order to understand the nation-wide magnitude and nature of genetic diversity of the species as a first step to establish long-term conservation strategy, we previously investigated the sequence divergence, geographic variation, and population genetic structure of the species collected from several sandy beaches of Korean coasts (East Sea, West Sea, and South Sea) and a remote island Jeju (Wang *et al*., 2011). From the sequencing of a total of 24 *S. aterrimus* individuals collected over nine sandy dunes belonging to four Korean provinces resulted in moderate to low magnitude of sequence diversity, the presence of closely related haplotypes, and relatively high gene flow estimate.

In this study, we additionally sequenced another mitochondrial gene, COII, to confirm the previous findings from COI gene sequence, because COII gene sequence also is often utilized for the question of population genetic diversity (Finn *et al.*, 2007), species identification (Oshaghi, 2007), gene flow (Marquez and Krafsur, 2002), and population genetic structure (Krafsur, 2002).

Materials and Methods

Sampling, DNA extraction, primer, PCR, and sequencing

Samples used in this study are identical to those used in Wang *et al.* (2011), wherein a total of 24 individuals collected from 9 Korean localities were employed, excluding one individual (Fig. 1 and Table 1). Total DNA that was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) was used for amplification of a full-length mitochondrial COII gene, corresponding to 688 bp in the *S. aterrimus.* Primers for forward and reverse directions were designed at $tRNA^{Leu}$ and $tRNA^{Lys}$ gene, respectively, from the alignment of four complete mitochondrial genomes of Adephaga in Coleoptera (Sheffield *et al*.,

*To whom the correspondence addressed
College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea.
Tel: +82-62-530-2073; Fax: +82-62-530-2079;
E-mail: ikkim81@chonnam.ac.kr

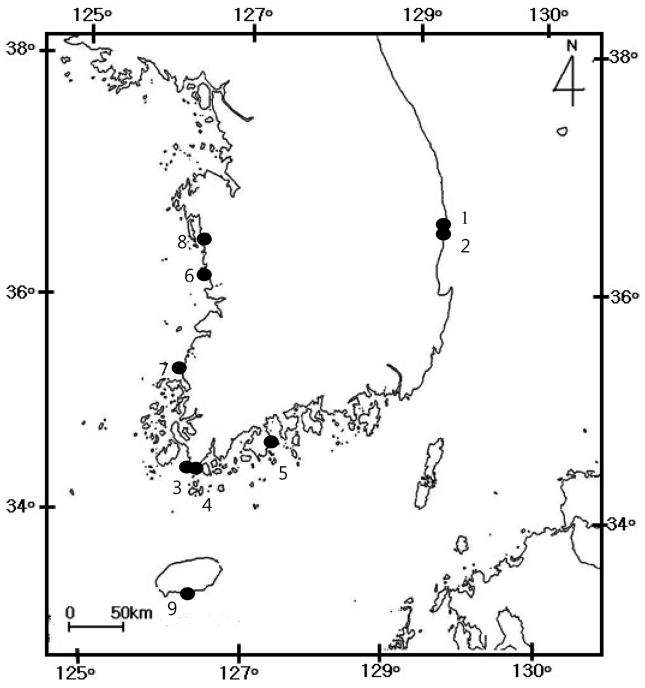

Fig. 1. Sampling location of *Scarites aterrimus* in Korea. General locality names are as follows: 1, Goraebul, Gyeongsangbukdo; 2, Duckcheon, Gyeongsangbukdo; 3, Songji, Jeollanamdo; 4, Buldeung, Jeollanamdo; 5, Namryul, Jeollanamdo; 6, Sohwang, Cungcheongnamdo; 7, Songseok, Jeollanamdo; 8, Jangpo, Chungcheongnamdo; and 9, Seowipo, Jejudo.

2008; Carmeron *et al*., 2009; Song *et al*., 2010; Pons *et al*., 2010). Internal primers were designed from *S. aterrimus* after a portion of COII was sequenced. The primer information is listed in Table 2. After an initial denaturation step at 94°C for 7 min, a 35-cycle amplification (94°C for 1 min, 48~53°C for 1 min, and 72°C for 1 min) was conducted. The final extension step was continued for 7 min at 72°C. To confirm the successful DNA amplification, electrophoresis was carried out using 0.5× TAE buffer on 0.5% agarose gel. The PCR product was then purified using PCR purification Kit (QIAGEN, Germany). DNA sequencing was conducted using the ABI PRISM® BigDye® Terminator ver. 3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer (PE Applied Biosystems, USA). All products were sequenced from both strands.

Sequence and phylogenetic analyses

Sequence alignment was performed using CLUSTAL X programs (ver. 1.8; Thompson *et al*., 1997). When homologous sequences from two individuals differed by ≥ one nucleotide base, the sequences were considered as different haplotypes. Haplotype designations were applied to new sequences as they were discovered (*i.e*., COIISA01, COIISA02, COIISA03 and so forth). Phylogenetic analysis was performed by maximum-parsimony

Table 1. A list of trapping localities, animal numbers, mitochondrial COII haplotypes of *Scarites aterrimus*

Collecting locality (no. of individuals)	Collection date	Animal number	COII haplotype	Genbank accession number
1. Sand dune of Goraebul, Byeonggok-ri	2009.07.22	SA3570	COIISA01	JQ780069
Gyeongsangbuk-do province (3)	2009.07.22	SA3571	COIISA01	JQ780070
	2009.07.22	SA3572	COIISA01	JQ780071
2. Sand dune of Dukcheon, Dukcheon-ri	2009.05.24	SA3573	COIISA01	JQ780072
Gyeongsangbuk-do province (1)				
3. Sand dune of Songji, Songho-ri	2008.07.22	SA3574	COIISA02	JQ780073
Jeollanam-do province (1)				
4. Sand dune of Buldeung, Tongho-ri	2008.07.22	SA3575	COIISA02	JQ780074
Jeollanam-do province (2)	2009.07.23	SA3576	COIISA02	JQ780075
5. Sand dune of Namryul, Namryul-ri	2009.05.23	SA3577	COIISA03	JQ780076
Jeollanam-do province (3)	2009.05.23	SA3578	COIISA01	JQ780077
	2009.05.23	SA3579	COIISA01	JQ780078
6. Sand dune of Sohwang, Sohwang-ri	2008.07.15	SA3580	COIISA01	JQ780079
Chungcheongnam-do province (6)	2008.07.15	SA3581	COIISA01	JQ780080
	2009.05.22	SA3582	COIISA01	JQ780081
	2009.05.22	SA3583	COIISA04	JQ780082
	2009.05.22	SA3584	COIISA01	JQ780083
	2009.05.22	SA3585	COIISA04	JQ780084

Table 1. Continued

Collecting locality (no. of individuals)	Collection date	Animal number	COII haplotype	Genbank accession number
7. Sand dune of Songseok, Songseok-ri	2009.05.22	SA3589	COIISA01	JQ780085
Jeollanam-do province (3)	2009.05.22	SA3590	COIISA01	JQ780086
	2009.05.22	SA3591	COIISA01	JQ780087
8. Sand dune of Jangpo, Jangpo-ri	2008.07.15	SA3592	COIISA01	JQ780088
Chungcheongnam-do province (1)				
9. Sand dune of Seogwipo, Seogwipo city	2004.05.18	SA3593	COIISA05	JQ780089
Jeju-do province (3)	2004.05.18	SA3594	COIISA01	JQ780090
	2004.05.18	SA3595	COIISA01	JQ780091

Table 2. List of pirmers used to amplify and sequence the COII sequences of *Scarites aterrimus*

Fragment	Primer name	Sequence (5` - 3`)
F1	COLCOIIF1	TCTAATATGGCAGAAT-AGTG
	SA-COII-INTER-R1	ATCTAATAGTCGAAAAT-CAG
F2	SA-COII-INTER-F2	TTGAAGCTAT-GAATACTCTG
	COLCOIIR1	GACCAATACT-TGCTTTCAG

(MP) method (Fitch, 1971) using PAUP* (Phylogenetic Analysis Using Parsimony and Other Method*) ver. 4.0b10 (Swofford, 2002). Unrooted tree was prepared using an equal weighting of transitions and transversions by heuristic search. The reliability of the trees was tested by 1,000 iterations of bootstrapping (Felsenstein, 1985). In order to further scrutinize the relationships among haplotypes one-step median networks, which provide insight into probable relationships among closely related lineages was prepared (Bandelt *et al*., 1995).

Population genetic analyses

Genetic diversity and distance were analyzed for the populations presenting more than one haplotypes. Thus, three populations (localities 5, 6, and 9) were subjected to these analyses. The maximum sequence divergence within population was obtained by extracting the estimate of unrooted pairwise distance within each population from PAUP (Swofford, 2002). Genetic distance and migration rate were estimated from mitochondrial DNA sequences and subroutines in the Arlequin ver. 3.0 (Excoffier *et al.*, 2005). Detailed methods are presented in Wang *et al.* (2011).

Results and Discussion

Sequence analysis

From 23 individuals of *S. aterrimus* collected over nine Korean localities a total of 5 haplotypes (COIISA01 – COIISA05) was obtained (Fig. 2). Four variable nucleotides were composed wholly of transitions (each two T↔C and G↔A) and only nucleotide position 118 caused amino acid replacements from His to Thr (Table 3). Pairwise comparison between pairs of haplotypes has shown sequence divergence ranging only from 0.145% to 0.291% (one ~ two nucleotides) (Table 4). This result indicates that COII gene is somewhat more conserved compared to COI gene in that COI sequence has provided ten haplotypes ranged from 0.152% to 0.912% (one ~ six nucleotides) (Wang *et al.*, 2011). With regard to distribution COIISA01 showed the widest distribution, accounting for seven of nine localities, composed of 69.6% (16 of 23) of samples utilized in this study (Table 1). Except for this haplotype the others are distributed either two (COIISA02) or one locality (COIISA03, COIISA04, and COIISA05) as a three (COIISA02) or one individual (Table 1). Concordantly to previous study by COI gene sequence, the distribution of *S. aterrimus* haplotypes can be summarized as a restricted local distribution in most haplotypes, except for one haplotype.

Phylogenetic and network analyses

Due to the limited variation among haplotypes (0.145% to 0.291%) phylogenetic relationships among 5 haplotypes are very poorly distinguished among them and the nodal support for each branch was nearly unsupported (Fig. 3). In the case of network analysis, COIISA01 was centered with the others branched off from this haplotype, evidencing “star phylogeny” (Fig. 4). Previously, such star phylogeny in the network tree was expected to be found, where widespread older haplotypes are located in the center of the network and recently derived

```
                                        30                                  60
COIISA01  ATGGCAACAT  GATCTAACCT  GAATCTTCAG  GACAGAGCCT  CTCCATTAAT  AGAACAATTG
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                        90                                 120
COIISA01  ACTTTCTTTC  ATGACCATAC  ATTAATAATT  TTAACGATAA  TTACAGTATT  AGTGGGGCAT
COIISA02  ..........  ..........  ..........  ..........  ..........  .......T..
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       150                                 180
COIISA01  GTAATATTAA  CACTGACCTA  TAATAAGAAT  ATTAATCGCT  ATCTTCTAGA  AGGACAATTA
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  .......T..  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ......A...  ..........  ..........  ..........
                                       210                                 240
COIISA01  ATTGAAGTAA  TTTGAACTAT  TCTTCCTGCT  ATTACACTTA  TTTTTATTGC  ACTTCCATCA
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       270                                 300
COIISA01  TTAAAATTAT  TATATTTACT  TGACGAAATT  AATAACCCTT  TAGTAACTTT  AAAATCAATT
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       330                                 360
COIISA01  GGTCATCAAT  GATATTGAAG  CTATGAATAC  TCTGATTTTA  GAAAAACAGA  ATTCGATTCA
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  .G........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       390                                 420
COIISA01  TACATAACAC  CTACAAATGA  ATTAAAAAAT  TCTGATTTTC  GACTATTAGA  TGTTGATAAT
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       450                                 480
COIISA01  CGAGTAATTT  TACCATTTAA  TAGTCAAATT  CGAGTATTAG  TAACAGCTAC  TGATGTTATT
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       510                                 540
COIISA01  CATTCATGAA  CAGTTCCTGC  CTTAGGGGTT  AAAATTGATG  CAACACCCGG  TCGGCTAAAT
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       570                                 600
COIISA01  CAAACAAGAT  TCTTTATAAA  TCGACCAGGA  TTATTTTTTG  GACAATGTTC  AGAAATCTGT
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........
                                       630                                 660
COIISA01  GGAGCTAACC  ACAGATTTAT  ACCTATTGCA  ATTGAAAGAG  TACCTACTAA  TATGTTTGTA
COIISA02  ..........  ..........  ..........  ..........  ..........  ..........
COIISA03  ..........  ..........  ..........  ..........  ..........  ..........
COIISA04  ..........  ..........  ..........  ..........  ..........  ..........
COIISA05  ..........  ..........  ..........  ..........  ..........  ..........

                                      688
COIISA01  AAATGAATAT  CTTCAATACA  AGAAAATT
COIISA02  ..........  ..........  ........
COIISA03  ..........  ..........  ........
COIISA04  ..........  ..........  ........
COIISA05  ..........  ..........  ........
```

Fig. 2. Sequencing alignment of ten five haplotypes (designed as COIISA01 – COIISA05) obtained from 688-bp of COII gene sequence from *Scarites aterrimus*. Only nucleotide positions that differ from haplotype COIISA01 are indicated.

Table 3. Type of substitution in the COII sequences of *Scarites aterrimus*

Nucleotide position	Nucleotide substitution	Amino acid substitution
118	C ↔ T	CAT (His) ↔ TAT (Thr)
138	C ↔ T	
147	G ↔ A	
312	A ↔ G	

Table 4. Pairwise comparisons among five haplotypes obtained from COII gene of *Scarites aterrimus*

	1	2	3	4	5
1. COIISA01	-	0.00145	0.00145	0.00145	0.00145
2. COIISA02	1	-	0.00291	0.00291	0.00291
3. COIISA03	1	2	-	0.00291	0.00291
4. COIISA04	1	2	2	-	0.00291
5. COIISA05	1	2	2	2	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

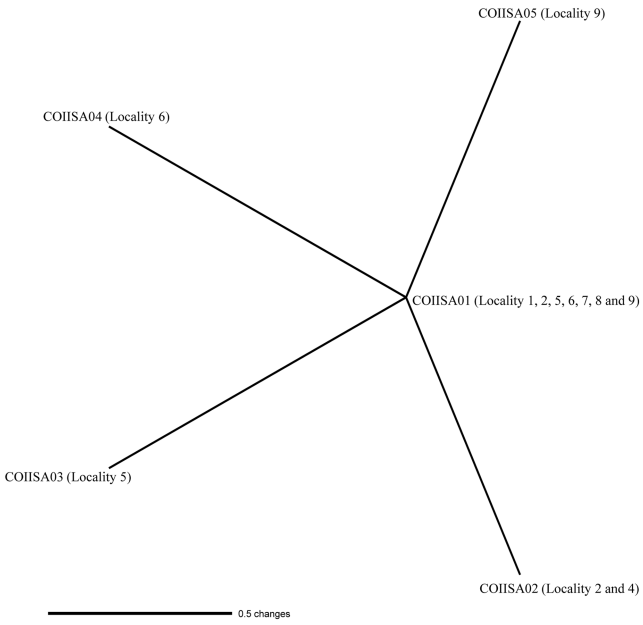

Fig. 3. Unrooted tree from 5 COII haplotypes of *Scarites aterrimus*. The tree was acquired via the MP method incorporated in PAUP (Phylogenetic Analysis Using Parsimony and Other) ver. 4.0b10 software (Swofford, 2002).

rare ones are stretched near the external nodes (Watterson and Guess, 1977). In this regard, the centered COIISA01 that was found in 7 of 9 localities (69.6%) (Table 1) might be the oldest haplotype and the remaining 4 haplotypes might be ones derived from

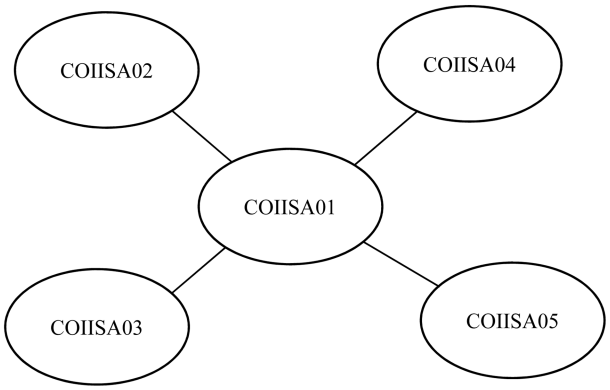

Fig. 4. Parsimonious one-step median networks analysis among 5 mitochondrial COII haplotypes of *Scarites aterrimus*. Each bar represents a single nucleotide difference from the neighboring haplotype.

COIISA01. This result, in turn, is interpreted that the *S. aterrimus* occurring in Korean peninsula did not experience historical biogeographic barriers that bolster genetic subdivision.

Population genetic analyses

Due to the limited samples and available haplotypes population genetic estimates were obtained only from locality 5 (Namryul), locality 6 (Sohwang), and locality 9 (Seogwipo). In a range of 0 ~ 1 in *H*, the sand dunes of Seogwipo (locality 9) and that of Goraebul (locality 1) was highest as 1. In terms of nucleotide diversity (π), Sohwang and Seogwipo were slightly higher than that of the remaining Namryul, but only minor difference was observed from the analysis. Previous COI-based analysis also supported higher π as 0.008105 (Wang *et al*., 2011). As has been seen in COI-based analysis (Wang *et al.*, 2011), genetic distance (F_{ST}) and per-generation migration rates (*Nm*) between pairs of populations overall showed high gene flow estimates in the COII sequence.

Summarized, twenty-three individuals of *S. aterrimus* from 9 Korean localities has shown similar result obtained previously from COI gene sequence (Wang *et al*., 2011) in that *S. aterrimus* occurring in Korean peninsula are well connected to each other with a high gene flow estimate, which indicates presence of no long-term zoogeographic barriers for the species. Comparison between two genes indicates that COI is slightly higher in sequence divergence, number of haplotypes, and resultant population estimates. For further scrutinized analysis for population perspective, further sensitive molecular marker, such as microsatellite DNA might be essential.

Table 5. Within-locality diversity estimates of *Scarites aterrimus*

Locality	SS[a]	NH[b]	*H*[c]	NP[d]	MSD[e] (%)	MPD[f]	π[g]
1. Goraebul	3	1	0.0000	0		-	-
2. Dukcheon	1	1	1.0000	0	-	-	-
3. Songji	1	1	1.0000	0	-	-	-
4. Buldeung	2	1	0.0000	0	-	-	-
5. Namryul	3	2	0.6667	1	0.145	0.666667	0.000969
6. Sohwang	6	2	0.5333	1	0.145	0.533333	0.000775
7. Songseok	3	1	0.0000	0	-	-	-
8. Jangpo	1	1	1.0000	0	-	-	-
9. Seogwipo	3	2	0.6667	1	0.145	0.666667	0.000969

[a]Sample size
[b]Number of haplotypes
[c]Haplotype diversity
[d]Number of polymorphic sites
[e]Maximum sequence divergence
[f]Mean number of pairwise differences
[g]Nucleotide diversity
-, unavailable.

Table 6. Fixation indices (F_{ST}) and migration rate (*Nm*) between pairs of populations of *Scarites aterrimus*

	6	9
5. Namryul	F_{ST} = 0.12195	F_{ST} = 0.0000
	Nm = 3.60000	*Nm* = 4503599627370496.00000
6. Sohwang		F_{ST} = 0.12195
		Nm = 3.60000
9. Seogwipo		

*$p < 0.05$.
inf, infinite.

Acknowledgement

This research was supported by the project "Eco Technopia 21" from the Ministry of Environment of Korea (052-091-074 [2009-2011]).

References

Bandelt HJ, Forster P, Sykes BC, Richards MB (1995) Mitochondrial portraits of human populations using median networks. Genetics 141, 743-753.

Cameron SL, Sullivan J, Song H, Miller KB, Whiting MF (2009) A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. Zool Scr 38, 575-590.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0. An integrated software package for population genetics data analysis. Evol Bioinf Online 1, 47-50.

Felsenstein J (1985) Confidence limits on phylogenics: an approach using the bootstrap. Evolution 29, 783-791.

Finn DS, Blouin MS, Lytle DA (2007*)* Population genetic structure reveals terrestrial affinities for a headwater stream insect. Freshwater Biol 52, 1881-1897.

Fitch WM (1971) Toward defining the course of evolution: minimal change for a specific tree topology. Syst Zool 20, 406-416.

Kawakami K, Sugiura S (2006) Feefing of a shore-inhabiting ground beetle, *Scarites aterrimus* (Coleoptera: Carabidae). Coleopterists Bull 60, 75-79.

Kim JI (1980) Deux nouvelles espèces du genre *Psammdius* Coréen (Col. Aphodiidae). The Korean J Entomology 5, 9-11.

Kim JI (2003) Insects Fauna from the Coastal Sand-dunes of Korea. Kor J Nature Conser 1, 27-45.

Krafsur ES (2002) Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities Insect Mol Biol 11, 37-45.

Marquez JG, Krafsur ES (2002) Gene flow among geographically diverse housefly populations (*Musca domestica* L.): A worldwide survey of mitochondrial diversity. J Hered 93, 254-259.

Oshaghi MA, Shemshad K, Yaghobi-Ershadi MR, Pedram M, Vatandoost H, Abaie MR, Akbarzadeh K, Mohtarami F (2007) Genetic structure of the malaria vector *Anopheles superpictus* in Iran using mitochondrial cytochrome oxidase

(COI and COII) and morphologic markers: A new species complex? Acta Trop 101, 241-248.

Pons J, Ribera I, Bertranpetit J, Balke M (2010) Nucleotide substitution rates for the full set of mitochondrial protein-coding genes in Coleoptera. Mol Phylogenet Evol 56, 796-807.

Sheffield NC, Song H, Cameron SL, Whiting MF (2008) A comparative analysis of mitochondrial genomes in Coleoptera (Arthropoda: Insecta) and genome descriptions of six new beetles. Mol Biol Evol 25, 2499-2509.

Song H, Nathan C, Sheffield NC, Cameron SL, Miller KB, Whiting MF (2010) When phylogenetic assumptions are violated: base compositional heterogeneity and among-site rate variation in beetle mitochondrial phylogenomics. Syst Entomol 35, 429-448.

Swofford DL (2002) PAUP* .Phylogenetic Analysis Using Parsimony (*and Other Method) ver. 4.10., Sinauer Associates, Sunderland, MA.

Thomson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24, 173-216.

Wang AR, Kim, MJ, Cho YB, Wan X, Kim I (2011) Geographic genetic contour of a ground beetle, *Scarites aterrimus* (Coleoptera: Carabidae) on the basis of mitochondrial DNA sequence. Int J Indust Entomol 22, 65-74.

Watterson GA, Guess HA (1977) Is the most frequent allele the oldest? Theor Popul Biol 7, 141-160.